[CONTRIBUTION FROM SECTION OF BIOCHEMISTRY, MAYO CLINIC]

Steroids Derived from Bile Acids. XIX. Barbier–Wieland Degradation in the 11-Keto Series[1]

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The degradation of methyl 3α, 9α-epoxy-11-keto-12α-bromocholanate (I) to 3α-acetoxy-11-ketoetiocholanic acid (XIII) by a combination of the Barbier–Wieland and Hoehn–Mason procedures is described. The etio acid was obtained in an over-all yield of 22%.

Prior to publication by Meystre and his associates[2] of their superior procedures for the degradation of the side chains of bile acids, the more laborious Barbier-Wieland method[3] was employed extensively in this Laboratory. By various modifications of published procedures, we were able to increase the yields of the intermediate compounds, and, despite the large number of steps involved, to achieve a satisfactory method for the preparation of derivatives of pregnan-20-one and of etiocholanic acid. Certain of these modifications appear to have general applicability, and we wish to report in the present paper our experience with bile acids of the 11-keto series.

Methyl 3α,9α-epoxy-11-keto-12α-bromocholanate (I) was available as starting material for this investigation. Conversion of this substance into methyl 3α-hydroxy-11-keto-12α-bromocholanate by cleavage of the epoxide ring has been described in a previous communication.[4] In practice, however, a satisfactory cleavage of the 3,9-cyclic ether was obtained at the noracid stage, and methyl 3α,9α-epoxy-11-keto-12α-bromocholanate (I) with the oxide bridge intact was accordingly employed in the first step of the degradation. Debromination at C-12 in a separate step was not necessary, since formation of the Grignard addition product was accompanied by complete removal of the bromine[5] at this position.

When a solution of I in dry benzene was slowly added to an ice-cold ethereal solution which contained an excess of phenylmagnesium bromide, a *homogeneous* reaction mixture was obtained, which was allowed to stand at 0°. The addition product, 3α,9α-epoxy-11-ketonorcholanyldiphenylcarbinol, was not usually isolated, but was dehydrated, in boiling acetic acid to the corresponding diphenylethylene, which was then oxidized with chromic acid to give 3α,9α-epoxy-11-ketonorcholanic acid (II) in an over-all yield of about 80% from I. Oxidation of the ethylene was carried out in a mixture of acetic acid, chloroform and water in the presence of sulfuric acid. Esterification with methanol which contained 1% of sulfuric acid furnished the methyl ester (III), in 96% yield. For cleavage of the oxide function the latter substance[6] was dissolved in a mixture of acetic anhydride and chloroform and treated at 0° in a sealed container with a large excess of hydrogen bromide. The resulting 3α-acetoxy-11-keto-12α-bromo derivative was re-esterified with concomitant loss of the acetyl group at C-3 and methyl 3α-hydroxy-11-keto-12α-bromonorcholanate (IV) was isolated in 87% yield by crystallization from chloroform–petroleum ether.

Degradation to the bisnor acid was carried out under conditions essentially the same as those described in the preceding paragraph. Addition of a solution of IV in benzene to an ethereal solution of the Grignard reagent resulted in separation of an insoluble complex, which, however, rapidly dissolved as the solution was stirred. After 42 hours at room temperature the Grignard complex was decomposed, and the diphenylcarbinol was extracted and simultaneously dehydrated and acetylated (C-3) in hot acetic anhydride. Oxidation followed by alkaline hydrolysis gave 3α-hydroxy-11-ketobisnorcholanic acid (V) in 74% over-all yield from IV. An additional 2% of V could be obtained by reprocessing the neutral material which remained after separation of the acidic product. Methyl 3α-hydroxy-11-ketobisnorcholanate (VI) was obtained in 96% yield by esterification of V in methanol which contained 8% of hydrogen chloride. The solution was boiled under a reflux for 7 hours.

Our experience with preparation of the diphenylcarbinol from VI indicated that this step does not proceed satisfactorily below room temperature or if an insoluble complex is formed. Preliminary investigation of the addition of VI to phenylmagnesium bromide in benzene–ether, benzene, anisole and other solvents was not encouraging. In every instance a voluminous precipitate separated, and the yields of diphenylethylene obtained after dehydration were poor. However, these difficulties were circumvented when it was discovered that a mixture of benzene and N-ethylmorpholine was a satisfactory solvent; the precipitate which formed initially dissolved within 24 hours. After 3 to 4 days at room temperature, ice and acid were added, the diphenylcarbinol was extracted, extraneous volatile by-products (diphenyl and so forth) were removed with steam and the diphenylethylene was formed in boiling acetic acid. The product was treated with methanolic alkali to hydrolyze acetylated material and to remove unchanged bisnor ester (VI). Crystallization from methanol gave a 72% yield of 3α-hy-

(1) The work reported in this paper was carried out in 1943–1944.

(2) C. Meystre, H. Frey, A. Wettstein and K. Miescher, *Helv. Chim. Acta.*, **27**, 1815 (1944), and subsequent papers.

(3) (a) Heinrich Wieland, O. Schlichting and R. Jacobi, *Z. physiol. Chem.*, **161**, 80 (1926); (b) W. M. Hoehn and H. L. Mason, THIS JOURNAL, **60**, 1493 (1938); (c) see also W. P. Long, C. W. Marshall and T. F. Gallagher, *J. Biol. Chem.*, **165**, 197 (1946).

(4) R. B. Turner, V. R. Mattox, L. L. Engel, B. F. McKenzie and E. C. Kendall, *ibid.*, **166**, 345 (1946).

(5) E. P. Kohler and M. Tishler, THIS JOURNAL, **54**, 1594 (1932).

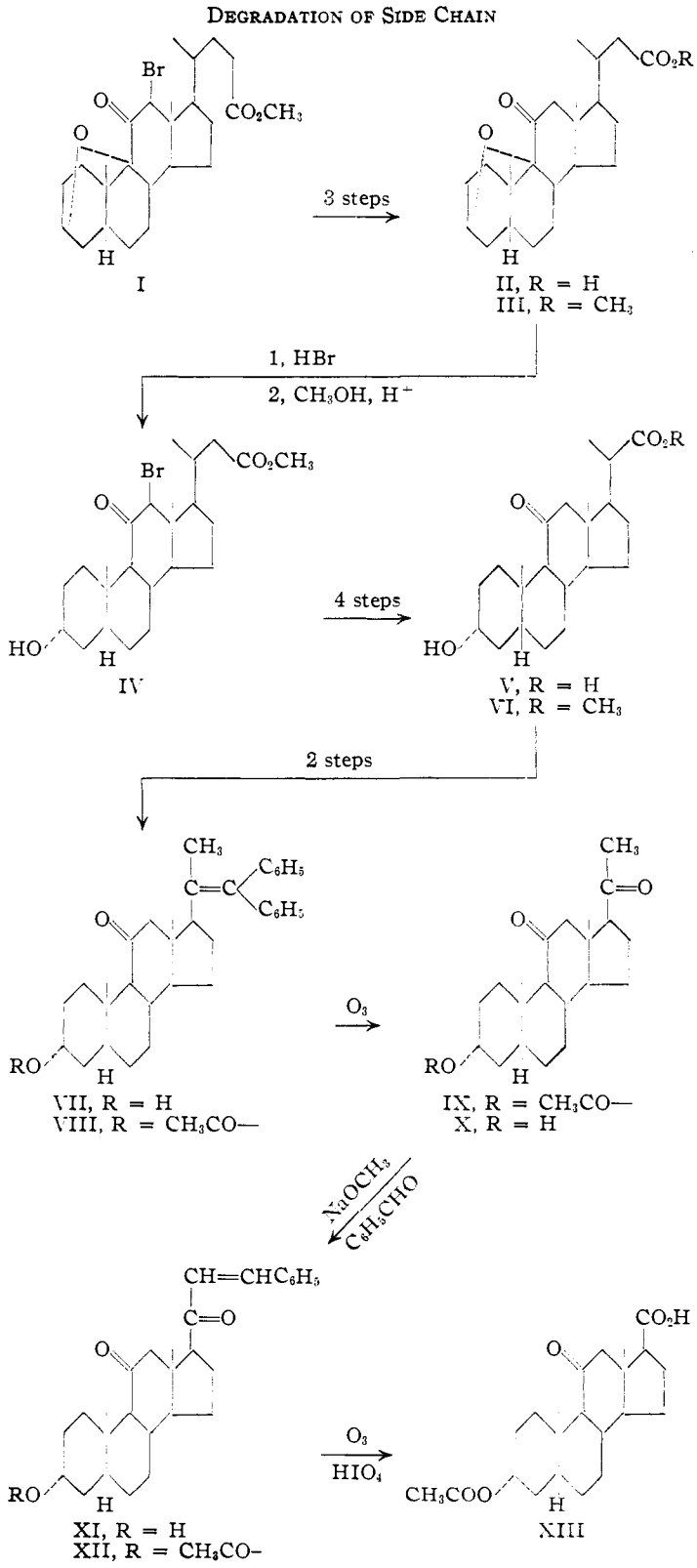
(6) The ester is more soluble than the free acid and was preferred for this reason.

droxy-11-keto-22,22-diphenyl-$\Delta^{20(22)}$-bisnorcholene (VII). The acetyl derivative (VIII) was obtained in nearly quantitative yield by acetylation of VII with acetic anhydride and pyridine.

Oxidation of the acetoxyethylene (VIII) with chromic acid was unsatisfactory, but ozonization[3b] proceeded smoothly when conducted in a mixture of ethyl acetate and methanol[7] at about −10°. The ozonide was decomposed with zinc and acetic acid in the cold, and benzophenone was removed by prolonged distillation with steam. By the use of this procedure 3α-acetoxypregnane-11,20-dione (IX) could be obtained consistently in yields of more than 80%. Ozonization of the free hydroxy compound (VII) under the same conditions afforded 3α-hydroxypregnane-11,20-dione (X), but the quality of the product was poor and purification was more difficult than was that of the acetyl derivative (IX).

Conversion of 3α-acetoxypregnane-11,20-dione (IX) into 3α-acetoxy-11-keto-etiocholanic acid (XIII) was accomplished by the procedure developed by Hoehn and Mason.[3b] A solution of IX in a minimal amount of methanol was treated with an excess of benzaldehyde in the presence of sodium methoxide. The benzylidene derivative (XI), which began to crystallize in a few minutes, was isolated after 12 hours in 95% yield. This material was then reacetylated (95% yield), and, after ozonization in ethyl acetate–methanol followed by treatment with periodic acid, gave 3α-acetoxy-11-ketoetiocholanic acid (XIII). This yield was 86%, and the over-all yield of XIII from methyl 3α,9α-epoxy-11-keto-12α-bromocholanate (I) was 22%.

In the course of this investigation several derivatives of bile acids not hitherto described in the literature were obtained. The preparative methods employed are unexceptional and therefore are not described in detail. Data for these compounds are listed in Table I.



Experimental[8]

3α,9α-Epoxy-11-ketonorcholanic Acid (II).—The conversion of methyl 3α,9α-epoxy-11-keto-12α-bromocholanate into 3α,9α-epoxy-11-ketonorcholanyldiphenylcarbinol[9] and 3α,9α-epoxy-11-keto-24,24-diphenyl-Δ^{23}-cholene[9] has been described. In the following procedure the intermediate carbinol and ethylene were not isolated. A solution of methyl 3α,9α-epoxy-11-keto-12α-bromocholanate (104.5 g.) in 700 ml. of benzene was added with shaking to 650 ml. of 1.85 molar phenylmagnesium bromide in ether. During the addition the flask, which was provided with a calcium chloride tube, was immersed in an ice salt bath and the temperature was kept below 5°. The reaction mixture was then stored at 0° under nitrogen. After 4 days[10] the solution was poured

(7) These conditions have since been successfully employed by Long, Marshall and Gallagher.[3c]

(8) All melting points were determined on the Fisher–Johns apparatus. We are indebted to Merck & Co., Inc., Rahway, New Jersey, and to Mr. William Saschek, Department of Biochemistry, Columbia University, for microanalyses. The yields reported in this paper represent that material which was satisfactory for use in subsequent experiments without further purification. Optical rotations were taken in chloroform (*c*, 1) at 27 ± 2° unless otherwise noted.

(9) V. R. Mattox and E. C. Kendall, *J. Biol. Chem.*, **188**, 589 (1950).

(10) It was later shown that formation of the Grignard reaction product proceeded very fast and was complete in 15 minutes.

TABLE I

NEW DERIVATIVES OF BILE ACIDS

Compound	Solvent[a]	M.p., °C.	$[\alpha]D$, °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
Methyl 3α-acetoxy-11-keto-12α-bromo-norcholanate	Chf.-P.e.	233–233.5	−5±2	61.05	60.88	7.69	7.87
				Br, 15.62	15.30		
3α-Acetoxy-11-ketonorcholanic acid	E.-P.e.	162.5–163.5	+78±2	71.74	71.62	9.15	9.11
3α-Hydroxy-11-ketonorcholanic acid	Dilute MeOH	253.5–255	+64±2[b]	73.36	73.10	9.64	9.58
3,11-Diketonorcholanic acid	Chf.-P.e.	202–204	+66±2	73.76	73.49	9.15	8.83
Methyl 3α-hydroxy-11-ketonorcholanate	E.-P.e.	124–124.5		73.81	74.05	9.81	10.10
3,11-Diketobisnorcholanic acid	E.-P.e.	208–209	+39±3	73.30	73.18	8.95	8.84

[a] Chf. = chloroform; E. = ether; P. e. = petroleum ether. [b] In methanol.

on a mixture of ice and 160 ml. of 12 *N* hydrochloric acid, and the aqueous phase was separated and extracted with benzene. Steam was passed through the organic phase until crystals formed and about 2.5 liters of water had condensed. The crystals were filtered off, dissolved in benzene, and the solution was dried by filtration through a pad of sodium sulfate and then concentrated to dryness. **Dehydration.**—The crude carbinol was dissolved in 1375 ml. of hot acetic acid and the solution was concentrated until about 100 ml. of distillate had been collected. The solution was then refluxed for 2.25 hours. **Oxidation.**—The solution of the crude diphenylethylene was cooled, 868 ml. of alcohol-free chloroform and 217 ml. of water were added and the flask was placed in a bath at 17°. While the mixture was being stirred mechanically, a solution of 217 ml. of 10.2 *N* chromic acid in 85% acetic acid was added at such a rate that the temperature remained below 20°. The solution was stirred for 2 hours at 18–20°, after which 217 ml. of 10 *N* sulfuric acid in 85% acetic acid was added in five equal portions at ten-minute intervals. The solution was stirred for an additional 2 hours at 18–20° and poured into about 7 liters of water. The organic phase was separated and the aqueous phase was extracted with two 500-ml. portions of chloroform. The chloroform solutions were combined, washed with water and concentrated to an oil. Crystals formed on addition of benzene; yield 66.4 g., m.p. 158–159°. The filtrate was concentrated to dryness, the residue was dissolved in ether and the ether solution was extracted three times with 0.5 *N* potassium hydroxide. The alkaline extracts were combined, acidified with hydrochloric acid and the solution was extracted with chloroform. The chloroform solution was concentrated to dryness and crystals (3.9 g., m.p. 157–158°) were obtained from benzene. The nor-acid separates from benzene with solvent of crystallization and usually melts with effervescence at about 110°, recrystallizes and remelts at about 157–159°. A sample of the acid which had been crystallized from benzene and air-dried overnight lost 9.18% at 100° and 0.1 mm.; calculated for 0.5 C_6H_6, 9.45%. The solvent-free product melted at 159.5–160° without previous softening; $[\alpha]D$ +90 ± 2°.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.84; H, 8.95.

Methyl 3α,9α-Epoxy-11-ketonorcholanate (III).—A solution of 70.3[11] g. of II in 1050 ml. of methanol which contained 10.5 ml. of concentrated sulfuric acid was refluxed for 2 hours, cooled and diluted with 192 ml. of water. The product which separated was filtered off and washed with water; yield: 62.16 g., m.p. 124.5–125.5°. The filtrate was concentrated to about 400 ml. and the crystals which separated were recrystallized from dilute methanol; yield 3.02 g., m.p. 123–124°. This represents a yield of 96% in the esterification and an over-all yield of 77.5% from I. The analytical sample, m.p. 125–125.5°, $[\alpha]D$ +87 ± 2°, was prepared by several recrystallizations from methanol.

Anal. Calcd. for $C_{24}H_{36}O_4$: C, 74.19; H, 9.34. Found: C, 74.52; H, 9.51.

Methyl 3α-Hydroxy-11-keto-12α-bromonorcholanate (IV).—Twenty grams of methyl 3α,9α-epoxy-11-ketonorcholanate (III), 25 ml. of chloroform and 25 ml. of acetic anhydride were placed in a bomb tube and cooled in a Dry Ice-bath. Dry hydrogen bromide (95 g.) was condensed in the reaction mixture and the tube was sealed. After 24 hours at 0° the tube was opened at Dry Ice temperature and the bulk of the hydrogen bromide was allowed to evaporate as the solution warmed to room temperature. After decomposition of the excess acetic anhydride with ice and water, the chloroform layer was separated, and the aqueous phase was extracted with chloroform. The chloroform solutions were combined, washed with water, dried over anhydrous sodium sulfate and diluted with 250 ml. of methanol, to which 5 ml. of acetyl chloride was added.[12] After 18 hours at room temperature, the solution was diluted with a large volume of water, the chloroform layer was separated, the aqueous phase was extracted with ether, and the combined organic phase was washed successively with dilute alkali, water and a saturated solution of sodium chloride. The solution was dried over anhydrous sodium sulfate, concentrated and diluted with petroleum ether. The product was obtained in three crops: I, 17.96 g., m.p. 155–156°; II, 2.50 g., m.p. 151–154°; III, 0.54 g., m.p. 150–153°; total crude yield, 21.00 g. (87%). Several recrystallizations from chloroform–petroleum ether furnished the analytical sample, m.p. 155–156°, $[\alpha]D$ −25 ± 2°.

Anal. Calcd. for $C_{24}H_{37}O_4Br$: C, 61.40; H, 7.95; Br, 17.02. Found: C, 61.46; H, 7.71; Br, 17.04.

3α-Hydroxy-11-ketobisnorcholanic Acid (V).—Methyl 3α-hydroxy-11-keto-12α-bromonorcholanate (IV) (60.63 g.) was dissolved in 650 ml. of dry benzene and added slowly to 650 ml. of an ether solution which contained 1.30 moles of phenylmagnesium bromide. During the addition the flask was cooled in an ice-bath. The voluminous precipitate which separated was dissolved by agitation. The flask was closed and allowed to stand at room temperature for 42 hours. The contents were poured over a mixture of ice and 400 ml. of concentrated hydrochloric acid, the aqueous phase was extracted with ether and the combined organic fractions were washed with dilute hydrochloric acid and water. The solvents were evaporated, and the residue subjected to a rapid current of steam. The solid phase was dissolved in ether, filtered through anhydrous sodium sulfate, concentrated and refluxed for 3 hours with 250 ml. of acetic anhydride. The material obtained after removal of the acetic anhydride under diminished pressure was dissolved in a mixture of 570 ml. of acetic acid, 280 ml. of alcohol-free chloroform, 85 ml. of water and 85 ml. of a 10 *N* solution of sulfuric acid in acetic acid. Addition of 140 ml. of a 9 *N* solution of chromic acid in 85% acetic acid was made at such a rate that the temperature of the reaction mixture did not rise above 65° and, after addition was complete, the solution was stirred for 35 minutes during which time the temperature fell to about 55°. A large volume of water was then added, and the aqueous phase was extracted twice with chloroform and once with ether. The organic fractions were combined, washed several times with water and extracted with 1 liter of 10% potassium hydroxide solution in 5 portions. The alkaline liquors were allowed to stand overnight at room temperature in order to remove the acetyl group at C-3, and acidified with dilute hydrochloric acid. The precipitate which formed was extracted with chloroform, the chloroform solution was dried, concentrated, and after dilution with petroleum ether the product was separated in 74% yield in 3 crops: I, 32.61 g., m.p. 231.5–233°; II, 1.79 g., m.p. 226–228°; III, 0.21 g., 224–226°. A sample for analysis was purified by several recrystallizations from acetone; m.p. 231–232° (reported[13] 195° and 223–225°), $[\alpha]D$ +41 ± 2°.

Anal. Calcd. for $C_{22}H_{34}O_4$: C, 72.89; H, 9.34. Found: C, 72.59; H, 9.70.

(11) The crystals contained 6.7% benzene.

(12) Karl Freudenberg and Wilhelm Jakob, *Ber.*, **74** [II], 1001 (1941).

(13) L. H. Sarett, *J. Biol. Chem.*, **162**, 601 (1946).

The neutral material which remained after extraction of the acidic oxidation product mentioned in the preceding paragraph was treated with methanol and acetyl chloride, and the benzophenone was removed by steam distillation. The residue (8.1 g.) was re-treated with phenylmagnesium bromide, dehydrated and oxidized by the procedure described in the preceding section. This afforded an additional 2.33 g. of 3α-hydroxy-11-ketobisnorcholanic acid, m.p. 227–229°, and increased the yield to 76.6%.

Methyl 3α-Hydroxy-11-ketobisnorcholanate (VI).—3α-Hydroxy-11-ketobisnorcholanic acid (V) (7.55 g.) was dissolved in 100 ml. of 8% methanolic hydrogen chloride and the solution was refluxed for 7 hours. After dilution with water and extraction with chloroform–ether, the organic phase was concentrated and the product was crystallized from chloroform–petroleum ether. Two crops, I, 7.24 g., m.p. 189–191°, and II, 0.27 g., m.p. 188.5–190°, were obtained (96%). The analytical sample, prepared by recrystallization from chloroform–petroleum ether, melted at 191.5–192° (reported[13] m.p. 193°), $[\alpha]_D$ +38 ± 2°.

Anal. Calcd. for $C_{23}H_{36}O_4$: C, 73.36; H, 9.64. Found: C, 73.46; H, 9.82.

3α-Hydroxy-11-keto-22,22-diphenyl-$\Delta^{20(22)}$-bisnorcholene (VII).—A solution of 30.89 g. of methyl 3α-hydroxy-11-ketobisnorcholanate (VI) in 75 ml. of dry benzene and 150 ml. of dry N-ethylmorpholine was added slowly to an ice-cold slurry of Grignard reagent prepared by dilution of 325 ml. of 2.48 molar phenylmagnesium bromide in ether with 150 ml. of benzene and 150 ml. of N-ethylmorpholine and removal of the ether under diminished pressure. A large amount of insoluble material was present at the end of the addition. After 3 hours at about 5° and 21 hours at room temperature the precipitate dissolved. During this interval the flask was shaken occasionally. At the end of 84 hours a mixture of ice and 250 ml. of concentrated hydrochloric acid was added, the aqueous phase was extracted with benzene, the benzene solution was washed with dilute hydrochloric acid and water, concentrated and volatile by-products were removed with a jet of steam. The residue was dried, dissolved in acetic acid and the solution was refluxed. After 3 hours the acetic acid was concentrated under reduced pressure, diluted with ether and washed with water. The ether was removed, the residue was taken up in 100 ml. of methanol and treated with a solution of 50 g. of potassium hydroxide in 200 ml. of methanol. After two days at room temperature, the mixture was diluted with a large volume of water extracted with ether. The ether extracts were washed with water, dried over anhydrous sodium sulfate and the ether was replaced with methanol on the steam-bath. The product was isolated in two crops, I, 24.93 g., m.p. 239.5–241°, and II, 3.45 g., m.p. 235–238° (72%). From the fraction obtained by acidification of the potassium hydroxide solution 1.20 g. of 3α-hydroxy-11-ketobisnorcholanic acid (V) was recovered. The diphenylethylene (VII), recrystallized from methanol and from benzene–petroleum ether, melted at 240–241°, $[\alpha]_D$ +270 ± 4°.

Anal. Calcd. for $C_{34}H_{42}O_2$: C, 84.60; H, 8.77. Found: C, 84.42; H, 8.99.

3α-Acetoxy-11-keto-22,22-diphenyl-$\Delta^{20(22)}$-bisnorcholene (VIII).—A sample of 24.28 g. of VII was acetylated at room temperature in 75 ml. of acetic anhydride and 75 ml. of pyridine. After 18 hours the material was separated in the usual way and crystallized from ether–petroleum ether. The product which separated (25.6 g., 97%) melted at 202–203.5°. Recrystallization from methanol gave crystals which were solvated, m.p. 132–135° (effervescence). The sample for analysis, m.p. 204–205°, $[\alpha]_D$ +266 ± 2°, was obtained by recrystallization from chloroform–petroleum ether.

Anal. Calcd. for $C_{36}H_{44}O_3$: C, 82.40; H, 8.45. Found: C, 82.63; H, 8.97.

3α-Acetoxypregnane-11,20-dione (IX).—A solution of 5.0 g. of 3α-acetoxy-11-keto-22,22-diphenyl-$\Delta^{20(22)}$-bisnorcholene (VIII) in 50 ml. of ethyl acetate and 50 ml. of methanol was cooled to −10° in a salt–ice-bath and treated with 3 molar equivalents of ozone. During the course of the reaction, when some of the starting materials crystallized out, the flow of ozone was stopped and the material was redissolved by stirring at about 0°. When the addition of ozone was complete, 3.0 g. of zinc dust and 20 ml. of 75% acetic acid were added, the mixture was stirred in the cooling bath until the starch–iodide test was negative (about 10 minutes), the excess zinc was filtered off and the solvents together with the by-product, benzophenone, were removed by steam distillation. The residue was dissolved in ether, and this solution was washed with dilute hydrochloric acid, water, dilute sodium hydroxide solution, dried, concentrated and diluted with petroleum ether. The product weighed 2.90 g. (81%) and melted at 127–128°. The analytical sample was recrystallized from ether–petroleum ether, m.p. 129.5–130° (reported[14] 132–133°), $[\alpha]_D$ +130 ± 2°.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 74.06; H, 9.37.

3α-Hydroxypregnane-11,20-dione (X).—Five grams of 3α-hydroxy-11-keto-22,22-diphenyl-$\Delta^{20(22)}$-bisnorcholene (VII) was ozonized as described in the preparation of IX. The product was obtained by crystallization from ether–petroleum ether in two crops: I, 2.60 g., m.p. 157–162°; II, 0.20 g., m.p. 155–158° (81%). A pure sample, m.p. 174–174.5° (reported[14] 172–174°), could be obtained only after several recrystallizations. The substance was identical with that obtained by hydrolysis of the acetyl derivative (IX).

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.56; H, 9.43.

3α-Hydroxy-21-benzylidenepregnane-11,20-dione (XI).—Ten grams of 3α-acetoxypregnane-11,20-dione (IX) was dissolved in 30 ml. of absolute methanol with warming. The solution was cooled to room temperature, and 6.8 ml. of freshly distilled benzaldehyde and 14.2 ml. of a 4.35 *N* solution of sodium methoxide in methanol were added. The container was sealed, allowed to stand overnight at 5° and the product which separated was filtered and washed with methanol; yield 10.29 g., m.p. 216.5–218.5°. Dilution of the mother liquor with water, extraction with ether and removal of the excess benzaldehyde in a current of steam gave an additional 0.35 g. of material, m.p. 212–215° (total crude product, 10.64 g., 95%). A sample prepared by recrystallization from benzene–petroleum ether melted at 220–221°, $[\alpha]_D$ +57 ± 2°.

Anal. Calcd. for $C_{28}H_{36}O_3$: C, 79.96; H, 8.63. Found: C, 80.33; H, 8.89.

21-Benzylidenepregnane-3,11,20-trione.—This compound was prepared as a derivative by oxidation of XI with a slight excess of chromic acid in chloroform–acetic acid at 0°. The trione melted at 190.5–192.5°, $[\alpha]_D$ +67 ± 2°, after recrystallization from benzene–petroleum ether.

Anal. Calcd. for $C_{28}H_{34}O_3$: C, 80.34; H, 8.19. Found: C, 80.20; H, 7.90.

3α-Acetoxy-21-benzylidenepregnane-11,20-dione (XII).—The 3α-hydroxybenzylidene derivative (XI) (4.48 g.) was acetylated by treatment with acetic anhydride and pyridine at room temperature. The product was isolated in the usual manner and was obtained by crystallization from chloroform–petroleum ether in two crops, I, 4.36 g., m.p. 205–207°, and II, 0.29 g., m.p. 197–199° (95%). The analytical sample melted at 205–206.5°, $[\alpha]_D$ +93 ± 2°.

Anal. Calcd. for $C_{30}H_{38}O_4$: C, 77.89; H, 8.28. Found: C, 77.94; H, 8.18.

3α-Acetoxy-11-ketoetiocholanic Acid (XIII).—Two molar equivalents of ozone was passed into a solution of 4.35 g. of 3α-acetoxy-21-benzylidenepregnane-11,20-dione (XII), dissolved in 75 ml. of ethyl acetate and 75 ml. of methanol cooled to −7° in a salt–ice mixture. The ozonide was decomposed in the cold with 4.0 g. of zinc dust, 20 ml. of acetic acid and 5 ml. of water, and after the starch–iodide test was negative, the mixture was filtered, the organic phase was washed with water, dried and concentrated under reduced pressure. The oily yellow residue was dissolved in 75 ml. of acetic acid and treated with 11.00 ml. of 7.35 *N* aqueous periodic acid at room temperature. After 3 hours the solution was diluted with a large volume of water, extracted with ether, the ether solution was washed with water and the steroid acid was extracted with ice-cold 1% aqueous sodium hydroxide solution. Acidification of the alkaline aqueous solution and extraction with ether afforded XIII, which was obtained from ether–petroleum ether in two crops: I, 2.42 g., m.p. 215–218°; II, 0.62 g., m.p. 212.5–216° (86%). An analytical sample, m.p. 220.5–

(14) J. von Euw, A. Lardon and T. Reichstein, *Helv. Chim. Acta*, **27**, 821 (1944).

222° (reported[13] m.p. 219–221°, reported[15] m.p. 223–225°), was obtained by crystallization from chloroform–petroleum ether.

Anal. Calcd. for $C_{22}H_{32}O_5$: C, 70.18; H, 8.57. Found: C, 70.34; H, 8.52.

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(15) J. von Euw, A. Lardon and T. Reichstein, *ibid.*, **27**, 1287 (1944).

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Steroids Derived from Bile Acids. XX. Degradation of 3α,9α-epoxy-11-Ketonorcholanic Acid to 3α,9α-Epoxy-11-ketoetiocholanic Acid[1]

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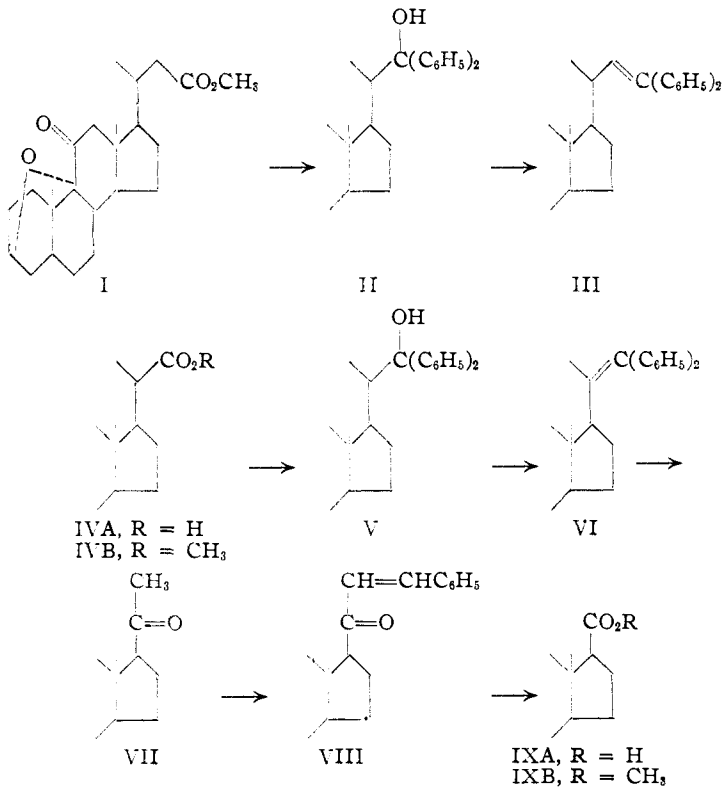
Methyl 3α,9α-epoxy-11-ketonorcholanate has been degraded to 3α,9α-epoxy-11-ketoetiocholanic acid by a combination of the Barbier–Wieland and Hoehn–Mason procedures in a yield of 36%.

Cleavage of the oxide ring in methyl 3α,9α-epoxy-11-ketocholanate with hydrogen bromide and degradation of the 3α-hydroxy steroid thus obtained to give 3α-acetoxypregnane-11,20-dione and 3α-acetoxy-11-ketoetiocholanic acid have been reported.[2] Degradation of the side chain was achieved by a modification of the Barbier–Wieland method, and the pregnane was converted into the etio acid by the Hoehn–Mason scheme. This paper describes the use of these procedures for conversion of methyl 3α,9α-epoxy-11-ketonorcholanate into 3α,9α-epoxy-11-ketoetiocholanic acid.



Presence of the 3,9-epoxide instead of the 3α-hydroxy group facilitated formation of the diphenylcarbinol derivatives, and since the cyclic ether was inert toward oxidation it was advantageous to degrade the side chain to the etio acid before restoration of the reactive hydroxyl group at C-3. However, in a study of the cleavage of the oxide ring at the nor, bisnor and etio ester stages it was found that there was a progressive decrease in the reactivity of the oxide ring toward hydrogen bromide.[3] The conditions which were satisfactory for the ester of 3α,9α-epoxy-11-ketonorcholanic acid failed to open the oxide completely when the ester of the etiocholanic acid was used; more severe conditions[3] led to formation of the 3,12α-dibromo-11-keto derivative of etiocholanic acid.

Experimental[4]

3α,9α-Epoxy-11-keto-23,23-diphenylnorcholan-23-ol (II).—A solution of 19.49 g. of methyl 3α,9α-epoxy-11-ketonorcholanate in 100 ml. of benzene was added slowly with mechanical stirring to 200 ml. of an ethereal solution of 1.5 *M* phenylmagnesium bromide in a flask immersed in a bath at −20°. The solution of steroid was added at such a rate that the temperature in the flask was 0 to 3°. The reaction mixture was stirred for 3 hours at 0°, the flask was sealed and stored in an ice-bath. After 24 hours the solution was poured over ice and ammonium chloride, the organic phase was washed and concentrated to dryness and the residue was crystallized from acetone–methanol. The volatile products were removed from the filtrate by steam distillation and more material was separated from acetone–methanol; total yield 81%. The analytical sample melted at 152–153°, $[\alpha]_D$ +61 ± 1°.

Anal. Calcd. for $C_{35}H_{44}O_3$: C, 81.99; H, 8.65. Found: C, 82.22; H, 8.55.

3α,9α-Epoxy-23,23-diphenyl-Δ^{22}-norcholen-11-one (III).—A solution of 5.39 g. of 3α,9α-epoxy-11-keto-23,23-diphenylnorcholan-23-ol in 10 ml. of acetic acid was refluxed for 1 hour and poured into water. The precipitate was collected, dried and recrystallized from acetone–methanol; wt. 5.02 g. (97%), m.p. 136–139°. The analytical sample melted at 136.5–137.5°, $[\alpha]_D$ +172 ± 2°.

Anal. Calcd. for $C_{35}H_{42}O_2$: C, 84.97; H, 8.56. Found: C, 85.12; H, 8.61.

(1) The work reported in this paper was carried out in 1943–1946.

(2) R. B. Turner, V. R. Mattox, W. F. McGuckin and E. C. Kendall, THIS JOURNAL, **74**, 5814 (1952).

(3) R. B. Turner, V. R. Mattox, L. L. Engel, B. F. McKenzie and E. C. Kendall, *J. Biol. Chem.*, **166**, 345 (1946).

(4) All melting points were determined on the Fisher–Johns apparatus. We are indebted to Merck & Co., Inc., Rahway, New Jersey, and to Mr. William Saschek, Department of Biochemistry, Columbia University, for microanalyses. Optical rotations were taken in chloroform ($c \sim 1$) at 27 ± 2°.